CARDIOPULMONARY SUPPORT AND PHYSIOLOGY

VASCULAR PERMEABILITY EFFECT OF ADENOVIRUS-MEDIATED VASCULAR ENDOTHELIAL GROWTH FACTOR GENE TRANSFER TO THE RABBIT AND RAT SKELETAL MUSCLE

Liubov Poliakova, MD^a
Imre Kovesdi, PhD^b
Xiatong Wang, MD^a
Maurizio C. Capogrossi, MD^{a,c}
Mark Talan, MD, PhD^a

Objective: Vascular endothelial growth factor has been used in preclinical studies and phase 1 and 2 clinical trials as a potent mediator of therapeutic angiogenesis; however, its ability to enhance the vascular permeability may be a source of potential complications. The objective of this work was to evaluate the effects of the intramuscular injection of an adenovirus vector coding for the 121-amino acid form of vascular endothelial growth factor (Ad.VEGF₁₂₁) on vascular permeability and edema development in rabbits and rats. **Methods:** Different concentrations of Ad.VEGF₁₂₁ ranging from 10⁵ to 10¹⁰ plaque-forming units/mL (3 × 10⁶–3 × 10¹¹ particles/mL) were injected into hind limb or forelimb muscles of Wistar rats or rabbits. The size of the scrotum, the circumferences of limbs, and the concentration of vascular endothelial growth factor in the serum were measured daily after injection. **Results:** The injection of different concentrations of Ad.VEGF₁₂₁ into the hind limb muscles of rabbits led to a dose-dependent scrotal edema in rabbits at concentrations higher than 10⁷ plaque-forming units/mL ($P = .002$). The edema developed slowly, reached its maximum level 6 days after the injection, and spontaneously resolved thereafter. At concentrations higher than 10⁹ plaque-forming units/mL the scrotal edema was accompanied by skin necrosis ($P = .0001$). No scrotal edema was observed in rats. **Conclusions:** The massive species-specific scrotal edema accompanied by skin ulceration and necrosis was observed only in rabbits treated with Ad.VEGF₁₂₁ in concentrations exceeding therapeutic doses. The therapeutic doses of Ad.VEGF₁₂₁ resulted in only moderate transient scrotal edema in rabbits, suggesting that the potential for side effects of vascular endothelial growth factor therapy as a result of increased vascular permeability should not be very alarming for generally healthy patients and may not cause a significant clinical problem in the treatment of peripheral vascular diseases. (J Thorac Cardiovasc Surg 1999;118:339-47)

Vascular endothelial growth factor (VEGF) gene transfer has been used to induce collateral blood vessel development and preserve blood flow to ischemic tissues in animal models of hind limb¹⁻⁴ and

cardiac⁵ ischemia. In light of the positive results obtained in these studies, clinical trials have been initiated in patients with critical limb ischemia⁶⁻⁸ and severe coronary artery disease.⁹⁻¹² Although definitive results on the therapeutic efficacy of these interventions are not yet available, it has been reported that some patients with severe peripheral vascular disease treated with the intramuscular injection of plasmid DNA encoding the 165-amino acid form of VEGF have had limb edema develop.⁷ This side effect was not unexpected because VEGF is known to enhance vascular permeability.¹³⁻¹⁷ However, gene therapy studies in animal models have failed to characterize this potentially clinically relevant side effect of VEGF gene transfer.

The objective of this work was to evaluate the effects

From the Gene Therapy Unit, Laboratory of Cardiovascular Science, Gerontology Research Center, National Institute on Aging, National Institutes of Health,^a Baltimore, GenVec Inc,^b Rockville, Md, and the Laboratorio di Patologia Vascolare, Istituto Dermatologico dell'Immacolata,^c Rome, Italy.

Received for publication Sept 15, 1998; revisions requested Jan 27, 1999; revisions received March 9, 1999; accepted for publication March 29, 1999.

Address for reprints: Mark Talan, MD, PhD, National Institute on Aging, Intramural Research Program, Gerontology Research Center, 5600 Nathan Shock Dr, Baltimore, MD 21224-6825.

12/1/98917

Fig 1. Edema of scrotum in rabbit. Representative example of time course of scrotal edema development in rabbit treated with intramuscular injection of 10^8 pfu Ad.VEGF₁₂₁ into right thigh. Panels show scrotum of same animal before injection of viral vector (A) and at 6 (B) and 12 days (C) after exposure to Ad.VEGF₁₂₁. Scales are in centimeters.

of the intramuscular injection of an adenovirus vector coding for the 121-amino acid form of VEGF (Ad.VEGF₁₂₁) on vascular permeability and edema development in rabbits and rats. The results show that Ad.VEGF₁₂₁ gene transfer to the skeletal muscle exerts both local and systemic effects to transiently enhance vascular permeability in the rabbit and that this response is delayed several days with respect to increases in VEGF serum level. Further, the development of edema is species dependent and is not observed in the rat.

Methods

Replication-deficient adenovirus vectors. Ad.VEGF₁₂₁ and the control vector (Ad.Null) have been previously described¹⁸ and were supplied by GenVec (GenVec, Inc, Rockville, Md). The viral vectors were stored in dialysis buffer at -70°C . Solutions for intramuscular injection were prepared immediately before use.

Animals. The experimental protocol was approved by the Animal Care and Use Committee of the Gerontology Research Center. Twenty-six male New Zealand White rabbits weighing 4.0 ± 0.5 kg (Human Research Products Inc Rabbitry, Denver, Pa) and 14 male Wistar rats weighing 650 ± 24 g (from the Gerontology Research Center's Wistar rat colony) were housed individually with constant access to food (Purina Lab Rabbit Chow Diet [Ralston Purina Company, St Louis, Mo] for rabbits and NIH-07 formula [National Institutes of Health] for rats) and water. The temperature in the vivarium was maintained at $22.5^{\circ}\text{C} \pm 1^{\circ}\text{C}$ with a 12-hour light/12-hour dark photocycle in which the light came on at 6 AM.

Experimental design. In all experiments rabbits were

placed under general anesthesia with subdermal injection of 5 mg/kg xylazine and 40 mg/kg ketamine. Studies were carried out according to the following experimental protocols.

Experimental protocol 1. Ad.VEGF₁₂₁ was injected in the muscles of the thigh of rabbits at 4 sites (0.25 mL per injection) along the projection of the femoral artery. Six concentrations of Ad.VEGF₁₂₁ (10^5 , 10^6 , 10^7 , 10^8 , 10^9 , and 10^{10} plaque-forming units (pfu/mL) and 2 concentrations of Ad.Null (10^6 and 10^8 pfu/mL) were used. Each concentration was injected into 2 animals. The size of the scrotum (area in square centimeters) was measured before the injection and daily during the 12 days after injection.

Experimental protocol 2. Rabbits received 4 intramuscular injections of Ad.VEGF₁₂₁ (0.25 mL per injection) into the forelimb at the concentration 10^{10} pfu/mL. The size of the scrotum and circumference of each forelimb were measured before injection and daily during the 12 days after injection. Blood (0.5 mL) was withdrawn daily from the marginal ear vein for quantification of the concentration of the 121-amino acid form of VEGF in the serum by enzyme-linked immunosorbent assay.

Experimental protocol 3. Rats were placed under general anesthesia with 60 mg/kg ketamine and 10 mg/kg xylazine (intraperitoneally) and were injected with Ad.VEGF₁₂₁ (10^9 pfu/mL) into each of 4 sites (0.25 mL per injection) of the muscles of the thigh along the projection of the femoral artery. The animals were examined daily for signs of edema. Blood samples (0.3 mL) for the determination of the concentration of the 121-amino acid form of VEGF in serum were collected daily through a cardiac puncture with the rats under general anesthesia.

Experimental protocol 4. The effects of Ad.VEGF₁₂₁ on vascular permeability in rabbits and rats were assessed with

Fig 2. Dose-response and time course of scrotal edema development in rabbits injected with Ad.VEGF₁₂₁ in right hind limb (experimental protocol 1). Two rabbits were injected with each concentration. Results are expressed as percentage of scrotal size before injection and each rabbit served as its own control. Data points represent mean.

the modified Miles assay.^{18,19} Because systemically injected Evans blue dye binds specifically with albumin, it accumulates in areas of increased vascular permeability. The change in local concentration of Evans blue dye in response to the intradermal injection of the investigated agent reflects the effect of this agent on permeability. The effect of the conditioned medium from human umbilical vein endothelial cells (American Type Culture Collection, Manassas, Va) cultures 24 hours after infection with Ad.VEGF₁₂₁ (100 pfu/cell) was compared with that of conditioned medium from human umbilical vein endothelial cells infected with Ad.Null (100 pfu/cell), uninfected cells, and saline solution. The concentration of VEGF in the conditioned medium of AdCMV.VEGF₁₂₁-infected human umbilical vein endothelial cells was 10⁴ ng/mL. Evans blue dye (60 mg/kg; Fisher Scientific Worldwide, Hampton, NH) was injected into the marginal ear vein of rabbits or intracardially in rats. Immediately after the injection of Evans blue dye the animals (6 rabbits and 6 rats) received the intradermal injection of 0.1 mL saline solution and 0.1 mL of each of the conditioned media. Other animals (3 rats and 3 rabbits) received the intradermal injection of 0.1 mL histamine (10⁵ ng/mL; Sigma, St Louis, Mo). All animals were killed 30 minutes after injection. The skin from the areas of local injections was cut out with a standard steel punch (1.0 cm in diameter) and placed into 4.0 mL formamide solution (Fisher Scientific) at 65°C for 36 hours to extract the dye. After filtration with glass filter (Pall Gelman Laboratory, Ann Arbor, Mich), the optical density of the filtrate was measured at 620A on a spectrophotometer (Beckman DU-50; Beckman Coulter, Inc, Fullerton, Calif).

Enzyme-linked immunosorbent assay. Enzyme-linked immunosorbent assay for quantification of the concentration of the 121-amino acid form of VEGF in the serum was carried out with human VEGF kit (Quantikine; R&D Systems, Minneapolis, Minn). After withdrawal blood samples were transferred from the syringe to collection tubes. The samples were kept for 30 minutes at room temperature for clot formation and then spun at 4°C for 30 minutes at 10,000 rpm. The serum from each tube was immediately frozen at -70°C. The assay procedure was carried out according to the supplier's instructions. The concentration of VEGF was determined on a spectrophotometer (Beckman DU-50) with a microtiter plate reader set at the absorbance of 450 nm.

Statistical analyses. Results were evaluated with analyses of variance for repeated measures (experimental protocols 1-3). Between factors were different concentrations of Ad.VEGF₁₂₁ (protocol 1) or different sites of injection (protocol 2); the within factor was time (in days) after treatment. Where appropriate the relative significance of difference between groups was determined by analyses of simple effects. The results of experimental protocol 4 were analyzed by *t* test. *P* < .05 was accepted as statistically significant.

Results

Experimental protocol 1. Rabbits treated with intramuscular injection of Ad.VEGF₁₂₁ in the hind limb had scrotal edema develop (Fig 1) without gross evidence of inflammation at the site of injection. The increase in the size of the scrotum and the time courses of this

Fig 3. Representative example of scrotal ulceration in rabbit 10 days after intramuscular injection of 10^{10} pfu Ad.VEGF₁₂₁. Scale is in centimeters.

effect in response to different concentrations of Ad.VEGF₁₂₁ are shown in Fig 2. Average scrotal size before the intramuscular injection of Ad.VEGF₁₂₁ was 7.5 ± 0.2 cm² (mean $\pm$ SE, $n = 12$). The edema started on day 3 after injection, reached its peak on days 6 and 7, and resolved spontaneously thereafter. There was no significant edema at concentrations of 10^5 and 10^6 pfu/mL of Ad.VEGF₁₂₁ (simple effect $P = 1.0$ and $P = .2$ for each concentration, respectively). At higher concentrations statistically significant edema developed in all animals and the time course of this effect was similar among groups (simple effects ranging from $P = .002$ for 10^7 pfu to $P = .00001$ for 10^{10} pfu). Furthermore, the magnitude of the effect was dose dependent, ranging from 2-fold at 10^7 pfu to 4.5-fold at 10^{10} pfu ($P = .004$ for group effect, $P = .00001$ for time effect, and $P = .0001$ for interaction). In the animals treated with concentrations of 10^9 and 10^{10} pfu/mL the edema was followed by skin necrosis in the scrotum (Fig 3). No local inflammatory reactions and no signs of edema were observed in rabbits treated with Ad.Null.

Experimental protocol 2. In additional experiments (experimental protocol 2) it was examined whether the edema was due to a systemic or localized effect of

Ad.VEGF₁₂₁. In these studies rabbits were injected with 10^{10} pfu/mL Ad.VEGF₁₂₁ (4 injections of 250 μ L each) in the forelimb and the size of the scrotum was measured in the ensuing days. In these experiments the scrotum did not increase in size progressively after the injection; rather, the edema developed abruptly on day 6 after the injection. (Fig 4). Average scrotal size before the intramuscular injection of 10^{10} pfu of Ad.VEGF₁₂₁ was 8.9 ± 1.2 cm² (mean $\pm$ SE, $n = 3$). The edema was less marked after injection in the forelimb than in the hind limb, with 2-fold and 4-fold increases in the size of the scrotum, respectively, and was not accompanied by skin necrosis. Measurements of the circumferences of the forelimbs are presented in Fig 5. Left and right limb circumferences were similar, and average limb circumference before the intramuscular injection of 10^{10} pfu Ad.VEGF₁₂₁ was 11.3 ± 0.1 cm (mean $\pm$ SE, $n = 6$). The circumferences of both forelimbs were enhanced on day 6 after injection (significant group and time effects and interaction, $P = .001$). The injected limb was more edematous than the contralateral limb ($137.2\% \pm 8.2\%$ on injected side and $107\% \pm 7.0\%$ on uninjected side with respect to preinjection size). Nevertheless, the edema on both limbs was sta-

Fig 4. Dose-response and time course of scrotal edema development in rabbits injected with 10^{10} pfu Ad.VEGF₁₂₁ in right forelimb (experimental protocol 3). Results are expressed as percentage of scrotal size before injection and each rabbit served as its own control. Data points represent mean; error bars indicate SE; n = 3.

tistically significant (simple effect $P = .001$ and $P = .005$ for injected and uninjected limbs, respectively).

Experimental protocol 3. The other experiments (experimental protocol 3) determined whether the effect of Ad.VEGF₁₂₁ to induce the edema was species specific and peculiar to the rabbit or also occurred in rats. Rats treated with intramuscular injections of Ad.VEGF₁₂₁ (10^9 pfu/mL) did not have any edema develop (n = 3) at any time within the 15 days after injection (data not shown).

The concentrations of VEGF in the serum after local intramuscular injection of Ad.VEGF₁₂₁ in rabbits (10^{10} pfu/mL, experimental protocol 2) and in rats (10^9 pfu/mL, experimental protocol 3) are shown in Fig 6, A and B, respectively. In both species the concentration of VEGF in the serum rose sharply on the second day after injection and was markedly reduced by the third day. It is noteworthy that the dose of Ad.VEGF₁₂₁ injected in the rats was 10-fold less than in the rabbits, to account for a body weight approximately 6-fold to 10-fold higher in the rabbit than in the rat.

Overall results. Taken together the results of this

study show that the intramuscular injection of Ad.VEGF₁₂₁ in the rabbit skeletal muscle exerts both local and systemic effects to induce the edema and suggest that this response may be species specific. This point was further addressed (experimental protocol 4) by performing a vascular permeability assay (Miles assay) in both rats and rabbits. The quantitative changes in local permeability in response to the intradermal injection of conditioned medium containing VEGF, medium from cells infected with Ad.Null, and medium from uninfected cells for rabbits and rats are presented in Fig 7. The increase in permeability in response to VEGF-containing medium was approximately twice as large in rabbits than in rats ($P = .01$). The response to the conditioned medium from cells infected with Ad.Null was not different from the response to the medium from uninfected cells for both rabbits and rats and was significantly lower than the response to medium containing VEGF ($P = .005$).

Additional experiments examined whether the enhanced vascular permeability in response to VEGF in rabbits in comparison with rats could also be

Fig 5. Dose-response and time course of forelimb edema development in rabbits injected with 10^{10} pfu Ad.VEGF₁₂₁ in right forelimb (experimental protocol 3). Results are expressed as percentage of forelimb circumference before injection and each forelimb served as its own control. Data points represent mean; error bars indicate SE; n = 3.

Fig 6. Time course of levels of 121-amino acid form of VEGF in sera of rabbits injected with 10^{10} pfu Ad.VEGF₁₂₁ in forelimb (A, n = 3) and rats injected with 10^9 pfu Ad.VEGF₁₂₁ in hind limb (B, n = 6). Data points represent mean; error bars indicate SE.

observed after the intradermal injection of histamine, another substance known to increase vascular permeability. Similarly to the effects of VEGF, the intrader-

mal injection of 0.1 mL of a solution containing 10^5 ng/mL histamine caused twice as large a response in rabbits as in rats ($P = .05$). Thus it appears that rats are

Fig 7. Effects of 121-amino acid form of VEGF in vascular permeability assay in rabbits and rats. Interventions were intradermal injection of 0.1 mL conditioned medium containing 10^4 ng/mL 121-amino acid form of VEGF (V), uninfected cells (UNINF), or cells infected with Ad.Null (NULL). Boxes represent mean; error bars indicate SE; n = 6.

less likely than rabbits to have an increase in vascular permeability develop in response to both VEGF and histamine.

Discussion

VEGF, also known as *vascular permeability factor*, is an endothelial cell-specific mitogen that induces angiogenesis and vascular permeability in vitro and in vivo. Although the angiogenic properties of VEGF have been reported in several successful preclinical animal studies^{1-5,14,20-22} and some phase 1 and 2 clinical studies,⁷ little attention has been paid to its ability to enhance vascular permeability, which may be responsible for potentially clinically relevant side effects of VEGF therapy.^{15,17,23,24}

Our experiments showed that a single intramuscular administration of Ad.VEGF₁₂₁ caused the appearance of VEGF in systemic circulation in rabbits and in rats 1 day after injection and led to a dose-dependent edema

in rabbits that reached its maximal level 6 days after the injection. Additional experiments showed that the effect of VEGF on tissue permeability was stronger in rabbits than in rats.

The edema in rabbits was most marked on day 6 after a single injection of Ad.VEGF₁₂₁. In experiments in which animals received injections into the thigh muscles scrotal edema developed slowly, reaching its maximum on day 6. When the site of injection was remote from the scrotum (front limb), the scrotal edema appeared more abruptly on day 6 after injection. The different time courses of the scrotal edema after injection at different sites suggest that increase in permeability is initiated at the site of injection of Ad.VEGF₁₂₁. Permeability increases gradually, on day 6 reaching a degree that affects remote sites susceptible to the effect of increased permeability. This notion is compatible with the properties of adenoviral vectors, with which expression peaks within 7 days after infection.²⁵ The

increased concentration of VEGF in the serum on the first day is a temporary overexpression from the local intramuscular injection. After that the virus infects the cells without significant access to systemic circulation.

The increase of vascular permeability caused by VEGF^{16,17} is related to endothelial cell activation caused by synthesis and release of mediators of the prostaglandin family. In a variety of cell types the growth factor activates phospholipase A₂ and induces the release of arachidonic acid and platelet activating factor within minutes. Moreover, it has been found that VEGF-induced protein extravasation is dose and tissue dependent and is inhibited by a selective platelet activating factor receptor antagonist. VEGF binds specifically to the endothelial cell growth tyrosine kinase receptors but not to other cell types. However, the concentration of receptors is not homogeneous and may vary among different tissues. The luminal surfaces of bronchial and pulmonary arteries possess the highest density of VEGF-binding sites, blood vessels of the pancreas and small intestine show a strong affinity for VEGF, and the most vascularized renal papillae have the highest degree of binding.¹⁶ It has been shown that lower concentrations of VEGF induce extravasation in the trachea and pancreas and that in increased doses it affects vascular permeability in the bronchi and duodenum. However, extravasation of protein in the lung parenchyma, heart, liver, and spleen has not been associated with intravenous injection of either VEGF or platelet activating factor.

VEGF-induced vascular permeability is an enhancement of vesicular-vacuolar organelle activity, which plays an important role in transendothelial transport of macromolecules.¹³ Connolly²⁶ speculated that on binding of VEGF with its receptors cytoskeletal changes would lead to cell contraction and increased intracellular vascular permeability. Another study has shown that VEGF is able to induce fenestration in endothelial cells in vivo and to convert endothelium from a nonfenestrated type into a fenestrated type.²⁷ The increased vascular permeability during the growth of tumor vasculature leads to flow of the bulk of fluid into the surrounding extracellular space.²⁸ Physiologically this means an excessive enlargement of the extravascular space. The tropism of the edema toward the scrotum may be explained by particularities of scrotal anatomic structure. The scrotum in rabbits is isolated from the body and does not have strong tissue support. It is among the richest areas of connective tissue spaces and lymphatic supply, which facilitate the accumulation of fluid.

In recent experiments on angiogenic effects of Ad.VEGF₁₂₁ in a rabbit hind limb ischemia model, a

significant acceleration of restoration of blood flow was observed after treatment with concentrations of 10⁶ and 10⁸ pfu/mL (unpublished data). These concentrations produced insignificant or spontaneously reversible scrotal edema. The massive scrotal edema accompanied by skin ulceration and necrosis was observed only with concentrations that exceeded those required for the therapeutic effects. Because a single local administration of VEGF was efficacious in stimulating angiogenesis and because therapeutic doses of Ad.VEGF₁₂₁ resulted in only moderate transient scrotal edema in rabbits, we conclude that the potential for side effects of VEGF therapy as a result of increased vascular permeability are minimal in generally healthy organisms and may not cause a significant clinical problem for treatment of peripheral vascular diseases. We did not observe any obvious changes in the respiratory and renal function of animals injected with Ad.VEGF₁₂₁. Nevertheless, it is conceivable that these areas might be more vulnerable to treatment-related complications. In the case of patients with compromised heart function, the increased permeability caused by VEGF therapy should increase alertness for pulmonary edema.

REFERENCES

1. Bauters C, Asahara T, Zheng LP, Takeshita T, Bunting S, Ferrara N, et al. Recovery of disturbed endothelium-dependent flow in the collateral-perfused rabbit ischemic hindlimb after administration of vascular endothelial growth factor. *Circulation* 1995;91:2802-9.
2. Chleboun JO, Martins RN. The development of the collateral circulation in an animal model of lower limb ischemia. *Aust N Z J Surg* 1994;64:202-7.
3. Takeshita S, Pu LQ, Stein LA, Sniderman AD, Bunting S, Ferrara N, et al. Intramuscular administration of vascular endothelial growth factor induces dose-dependent collateral artery augmentation in a rabbit model of chronic limb ischemia. *Circulation* 1994;90(Pt 2):228-34.
4. Pu LQ, Sniderman AD, Brassard R, Lachapelle KJ, Graham AM, Lisbona R, et al. Enhanced revascularization of ischemic limb by angiogenic therapy. *Circulation* 1993;88:208-15.
5. Mack CA, Patel SR, Schwarz EA, Zanzonico P, Hahn RT, Ilcail A, et al. Biologic bypass with the use of adenovirus-mediated gene transfer of the complementary deoxyribonucleic acid for vascular endothelial growth factor 121 improves myocardial perfusion and function in the ischemic porcine heart. *J Thorac Cardiovasc Surg* 1998;115:168-76.
6. Isner JM, Walsh K, Symes J, Pieczek A, Takeshita S, Lowry J, et al. Arterial gene therapy for therapeutic angiogenesis in patients with peripheral artery disease. *Circulation* 1995;91:2687-92.
7. Isner JM, Pieczek A, Schainfeld R, Blair R, Haley L, Asahara T, et al. Clinical evidence of angiogenesis after arterial gene transfer of phVEGF165 in patient with ischaemic limb. *Lancet* 1996;348:370-4.
8. Isner JM, Walsh K, Symes J, Pieczek A, Takeshita S, Lowry J, et al. Arterial gene transfer for therapeutic angiogenesis in patients with peripheral artery disease. *Hum Gene Ther* 1996;7:959-88.

9. Crystal RG. Deficient adenovirus vector containing the VEGF121 cDNA to the ischemic myocardium of individuals with life-threatening diffuse coronary artery disease. In: Clinical trial. Registration No. 9711-221. New York: The New York Hospital-Cornell Medical Center; 1998.
10. Isner JM. Gene therapy for myocardial angiogenesis. In: Clinical trial. Registration No. 9802-088. Boston: Tufts University School of Medicine; 1998.
11. McCluskey T. VEGF: preliminary clinical studies in patients with coronary artery disease. In: Course angiogenesis & direct myocardial revascularization. Washington Hospital Center; Washington [DC]. 1998 Jun 5-6.
12. Symes J. VEGF: Status of clinical trail in peripheral vascular and myocardial disease. In: Course angiogenesis & direct myocardial revascularization. Washington Hospital Center; Washington [DC]. 1998 Jun 5-6.
13. Plate KH, Warnke PC. Vascular endothelial growth factor. *J Neur Oncol* 1997;35:365-72.
14. Kenneth AT. Vascular endothelial growth factor, a potent and selective angiogenic agent. *J Biol Chem* 1996;271:603-6.
15. Cheng SY, Nagane M, Huang HJ, Cavenee WK. Intracerebral tumor-associated hemorrhage caused by overexpression of the vascular endothelial growth factor isoforms VEGF121 and VEGF165 but not VEGF189. *Proc Natl Acad Sci U S A* 1997;94:12081-7.
16. Sirois MG, Edelman ER. VEGF effect on vascular permeability is mediated by synthesis of platelet-activating factor. *Am J Physiol* 1997;272:H2746-56.
17. Rizk B, Aboulgar M, Smitz J, Ron-El R. The role of vascular endothelial growth factor and interleukins in the pathogenesis of severe ovarian hyperstimulation syndrome. *Hum Reprod Update* 1997;3:255-66.
18. Muhlhauser J, Merrill MJ, Pili R, Maeda H, Basic M, Bewig B, et al. VEGF165 expressed by a replication-deficient recombinant adenovirus vector induces angiogenesis in vivo. *Circ Res* 1995;77:1077-86.
19. Takeuchi UK, Movat HV. Simple method for quantitation of enhanced vascular permeability. *Proc Soc Exp Biol Med* 1970;133:1384-7.
20. Stephan D, Nabel EG. Gene and other biological therapies for vascular diseases. *Fundam Clin Pharmacol* 1997;11:97-110.
21. Casscells W. Growth factor therapies for vascular injury and ischemia. *Circulation* 1995;91:2699-702.
22. Hudlicka O, Brown M, Egginton S. Angiogenesis in skeletal and cardiac muscle. *Physiol Rev* 1992;72:369-96.
23. Jeltsch M, Kaipainen A, Joukov V, Meng X, Lasko M, Rauvala H. Hyperplasia of lymphatic vessels in VEGF-C transgenic mice. *Science* 1997;276:1423-5.
24. Liu NF, He QL. The regulation effects of cytokines on lymphatic angiogenesis. *Lymphology* 1997;30:3-12.
25. Smith RC, Walsh K. Prospects for intravascular Gene therapy. *J Clin Apheresis* 1997;12:140-5.
26. Connolly D. Vascular permeability factor a unique regulator of blood vessel function. *J Cell Biochem* 1991;47:219-23.
27. Diaz-Flores L, Gutierrez R, Varela H. Angiogenesis: an update. *Histol Histopathol* 1994;9:807-43.
28. Symes JF, Sniderman AD. Angiogenesis: potential therapy for ischaemic disease [published erratum appears in *Curr Opin Lipidol* 1994;5(5):following table of contents]. *Curr Opin Lipidol* 1994;5:305-12.